OIPE JC169
OCT 30 2003
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Cybille Delacroix-Muirheid
Art Unit	:	1614
Applicants	:	Ben M. Dunn, Janet K. Yamamoto, Maki Arai
Serial No.	:	09/763,037
Filed	:	February 15, 2001
Conf. No.	:	2654
For	:	Combination Therapy for Treatment of FIV Infection

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JANET K. YAMAMOTO, Ph.D. UNDER 37 CFR §1.132

Sir:

I, Janet K. Yamamoto, Ph.D., hereby declare:

THAT, I am a co-inventor of the subject matter claimed in U.S. patent application Serial No. 09/763,037 (hereinafter the '037 application);

THAT, I have read and understood the '037 application;

THAT, I have read and understood the rejection of claims in the Office Action mailed June 26, 2003 in the '864 application;

AND, being thus duly qualified, do further declare:

The Examiner has rejected the claims of the subject application under 35 USC §112, first paragraph, as nonenabled by the subject specification. The Examiner asserts that the subject specification is not enabled for treatment of cats infected with strains of FIV other than FIV_{UK8}. I respectfully submit that the subject specification enables the claimed methods for treating cats infected with other strains of FIV.

Although there are sequence differences among the various strains of FIV, they have the same functional enzymatic activities, such as reverse transcriptase. Reverse transcriptase shares significant homology among the various strains of FIV. Attached as Exhibit A is a table

showing percent sequence identity of reverse transcriptase among various strains of FIV (from which FIV subtypes A, B, and C are represented). For example, as shown in the table, reverse transcriptase has about 96% sequence identity between FIV_{UK8} (an FIV subtype A) and FIV_{Pet} (an FIV subtype A) and about 88% sequence identity between FIV_{UK8} and FIV_{FC1} (an FIV subtype C) and FIV_{TM2} (an FIV subtype C). The lowest percentage sequence identity among all the strains shown in the table is only 87.3%. Thus, in view of the significant sequence identity for reverse transcriptase among FIV strains, the ordinarily skilled artisan, having the benefit of the teachings of the subject application, would have expected the methods of the subject invention could be used to treat a cat infected with any strain of FIV.

Also submitted herewith as Exhibit B is data showing a reduction of FIV replication in infected cells *in vitro* following treatment with AZT and 3TC. Feline T-cell lines chronically infected with FIV_{Pet}, FIV_{Bang}, or FIV_{Shi} at $2x10^5$ cells/ml were treated with AZT, 3TC, or AZT/3TC combination. Drug doses that were used in these studies were nontoxic to the cells. Culture supernatants were harvested every 3 days and cells were suspended with fresh culture media containing the appropriate drug(s). Viral replication was determined by measuring the levels of viral reverse transcriptase activity in the culture supernatants. The peak reverse transcriptase titers detected in the culture supernatants of untreated infected cells were 1212479 cpm/ml (FIV_{Pet}), 306052 cpm/ml (FIV_{Bang}), and 974183 cpm/ml (FIV_{Shi}). The reverse transcriptase values are shown as a percentage of control values and are the result of triplicate cultures. These results show that treatment with an AZT/3TC combination reduces viral load in cells infected with FIV_{Pet} (subtype A), FIV_{Bang} (subtype B), and FIV (subtype D).

In addition, submitted herewith as Exhibit C is data showing that cats infected with FIV_{Bang} or FIV_{Pet} showed a significant decrease in virus load when treated with AZT and 3TC. Specific pathogen free (SPF) cats were inoculated with a high dose of 200 CID_{50} of respective FIV strains and were 13 weeks post-infection at the time of first treatment (0 wpt). Cats were treated daily for 5 weeks with a total of 10 mg/kg/day (5 mg each of drug per kg per day). The first day of treatment was on the first day of 0 wk post-1st treatment (0 wpt) which serves as the reference time-point for pre-, during, and post-treatment. Quantitative virus isolation (VI) was performed on peripheral blood mononuclear cells (PBMC) collected at 1 and 4 weeks before the first treatment (-4 and -1 wpt), at the end of the second and fourth weeks of treatment (2 and 4

wpt), and at 2 and 6 weeks after the last treatment (7 and 11 wpt). Immunoblot analysis was also performed to quantify anti-FIV antibody in cat sera.

In the FIV_{Bang}-infected cats, 3 of the 5 treated cats exhibited a decrease in VI and anti-FIV antibody (WB), indicative of a decrease in virus load in the cats following treatment. In contrast, none of the cats receiving placebo exhibited a decrease in WB and only 1 of 5 cats exhibited a decrease in VI. Similarly, in FIV_{Pet}-infected cats, 5 of 5 treated cats exhibited a decrease in VI, whereas only 1 of 5 cats receiving placebo exhibited a decrease in VI. Thus, it is clear from the data presented in Exhibit B that cats infected with FIV strains other than FIV_{UK8} can be successfully treated.

In conclusion, I respectfully submit that the claimed method of the '037 application is enabled for treating cats infected with any strain of FIV.

The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed: Janet K. Yamamoto

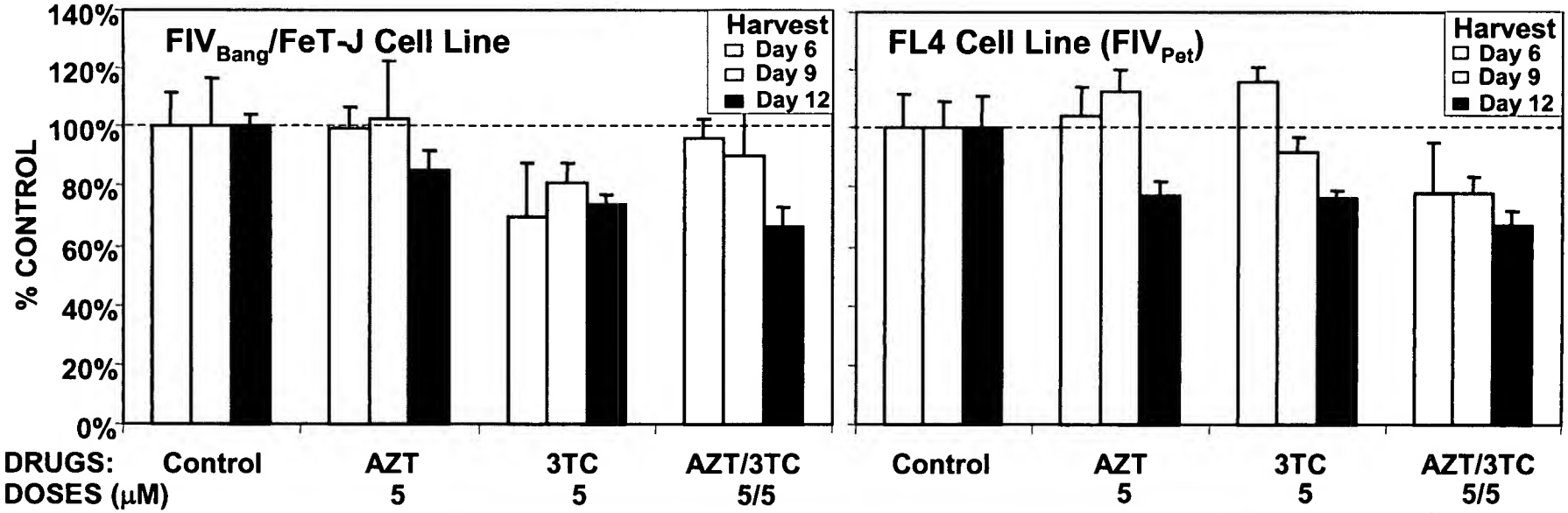
Janet K. Yamamoto, Ph.D.

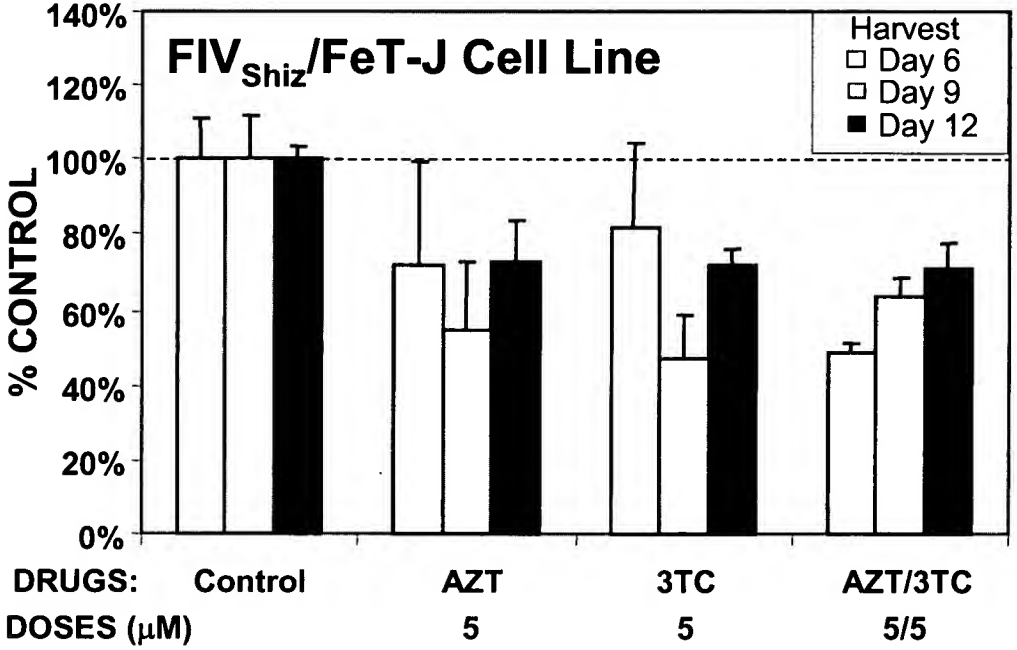
Date: October 23, 2003

% IDENTITY COMPARISON BETWEEN THE REVERSE TRANSCRIPTASE SEQUENCES OF FIV STRAINS

	Subtype A FIV-UK8	Subtype A FIV-Pet	Subtype A/B FIV-Bang*	Subtype B FIV-FC1	Subtype B FIV-TM2	Subtype C FIV-BM3070
FIV-UK8	100.0	95.8	96.2	88.6	88.4	92.0
FIV-Pet		100.0	97.3	87.7	87.3	91.1
FIV-Bang			100.0	88.2	88.1	91.0
FIV-FC1				100.0	94.6	90.4
FIV-TM2					100.0	89.0
FIV-BM3070						100.0

* FIV-Bang is a recombinant of subtype A *gag/pol* and subtype B *env*.


FIV$_{Bang}$/FeT-J Cell Line
FL4 Cell Line (FIV$_{Pet}$)
Harvest
Day 6
Day 9
Day 12
% CONTROL
140%
120%
100%
80%
60%
40%
20%
0%
DRUGS: Control AZT 3TC AZT/3TC
DOSES (μM) 5 5 5/5
Control AZT 3TC AZT/3TC
5 5 5/5


FIV$_{Shiz}$/FeT-J Cell Line
Harvest
Day 6
Day 9
Day 12
% CONTROL
140%
120%
100%
80%
60%
40%
20%
0%
DRUGS: Control AZT 3TC AZT/3TC
DOSES (μM) 5 5 5/5

Oral AZT/3TC Treatment of Chronically FIV_{Bang}-infected Cats

Group # - Cat #	Treatment (mg/kg/day)	Pretreatment -4 wpt to -1 wpt		Treatment 2 wpt	Treatment 4 wpt		Post-Treatment 7 wpt	Post-Treatment 11 wpt		Summary
		WB [b]	VI [c]	VI [c]	WB [b]	VI [c]	VI [c]	WB [b]	VI [c]	
A-3042	AZT	7	≥5	4	7	4.5	≥5	6	≥5	3 of 5 decrease in VI (red)
A-3058	+	4	≥5	≥5	4	≥5	≥5	3	≥5	3 of 5 decrease in WB (red)
A-3033	3TC	5	≥5	4	5	4	≥5	4	≥5	
A-3040		5	≥5	≥5	5	≥5	≥5	5	≥5	
A-3069		5	≥5	3	5	≥5	≥5	5	4	
B-3035	Placebo	6	≥5	≥5	6	≥5	≥5	6	≥5	1 of 5 decrease in VI (red)
B-3062		6	≥5	≥5	6	≥5	≥5	6	≥5	0 of 5 decrease in WB
B-3052		4	≥5	≥5	4	≥5	≥5	4	≥5	
B-3055		5	≥5	4	5	≥5	≥5	5	≥5	
B-3017		5	≥5	≥5	5	≥5	≥5	5	≥5	

Oral AZT/3TC Treatment of Chronically FIV_{Pet}-infected Cats

Group # - Cat #	Treatment (10mg/kg/day)	Pretreatment -4 wpt to -1 wpt		During Treatment 2 wpt	During Treatment 4 wpt		Post-Treatment 7 wpt	Post-Treatment 11 wpt		Summary
		WB [b]	VI [c]	VI [c]	WB [b]	VI [c]	VI [c]	WB [b]	VI [c]	
A-3PK4	AZT	3	**5**	3	3	4	3	3	4	5 of 5 decrease in VI (red) with no
A-3PB3	+	4	**5**	3	4	4	**5**	4	4	increase
A-3PJ4	3TC	4	**5**	**5**	4	4	3	4	**5**	
A-3PB5		4	≥5	≥5	3	3	4	3	4	1 of 5 decrease in WB (red) with no
A-3OZ1		5	**4**	3	5	2	**4**	5	2	increase
B-3PJ3	Placebo	3	**5**	4	4	4	4	4	**5**	1 of 5 decrease in VI (red) with 3 of 5
B-3PB1		3	**4**	**4**	4	≥5	**4**	4	**4**	increase in VI during treatment (blue)
B-3OZ4		5	**2**	**2**	5	≥5	**3**	5	**2**	
B-3PJ2		4	**4**	**4**	5	**4**	≥5	4	**4**	2 of 5 consistent increase in WB (blue)
B-3PC1		5	**2**	3	5	3	3	5	3	

[b] Immunoblot analysis (WB) was used to titrate anti-FIV antibodies in the serum and is shown as log value of the dilution whereby dilution of $1:10^3$ is 3.

[c] Quantitative virus isolation (VI) was performed to measure the virus load in peripheral blood mononuclear cells (PBMC).

VI of 1 = $5x10^6$ cells were the minimal number needed to detect FIV by virus isolation.
VI of 2 = $5x10^5$ cells were the minimal number needed to detect FIV by virus isolation.
VI of 3 = $5x10^4$ cells were the minimal number needed to detect FIV by virus isolation.
VI of 4 = $5x10^3$ cells were the minimal number needed to detect FIV by virus isolation.
VI of 5 = $5x10^2$ cells were the minimal number needed to detect FIV by virus isolation.